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Attorney Docket No.: SALK1520-2
(088802-8752)

1. (Amended) A method for modulating the expression of an exogenous gene in an isolated cell containing:

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- (i) a modified ecdysone receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to a response element, wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said response element; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and
 - (ii) a DNA construct comprising said exogenous gene under the control of said response element, wherein said response element (a) has about 12-20 base pairs, (b) binds to said modified ecdysone receptor, and (c) does not bind to farnesoid X receptor (FXR);

said method comprising providing to the cell an effective amount of one or more ligands for said modified ecdysone receptor; wherein said one or more ligands are not normally present in the cell; and wherein said one or more ligands are not toxic to said cell.

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4. (Amended) A method according to claim 1 wherein the DNA-binding domain of said modified ecdysone receptor is derived from a nuclear receptor.

5. (Amended) A method according to claim 1 wherein said activation domain is obtained from a nuclear receptor.

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11. (Amended) A method according to claim 1, wherein said silent partner is RXR.

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22. (Amended) A method of inducing the expression of an exogenous gene in an isolated cell containing:

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- (i) DNA encoding a modified ecdysone receptor under the control of an inducible promoter, wherein said modified ecdysone receptor, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to a response element, and wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said response element; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;
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- (ii) a DNA construct comprising said exogenous gene under the control of said response element, wherein said response element (a) has about 12-20 base pairs, (b) binds to said modified ecdysone receptor, and (c) does not bind to farnesoid X receptor (FXR); and
- (iii) one or more ligands for said modified ecdysone receptor;

said method comprising subjecting said cell to conditions suitable to induce expression of said modified ecdysone receptor.

23. (Amended) A method of inducing expression of an exogenous gene in an isolated cell containing a DNA construct containing said exogenous gene under the control of a response element, wherein said response element (a) has about 12-20 base pairs, (b) binds to said modified ecdysone receptor, and (c) does not bind to farnesoid X receptor (FXR), said method comprising introducing into said cell:

a modified ecdysone receptor, wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said response element; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain

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is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and

one or more ligands for said modified ecdysone receptor,

wherein said receptor, in combination with a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said response element, activating transcription therefrom.

24. (Amended) A method for the expression of a recombinant product detrimental to isolated host cells, said method comprising:

transforming suitable isolated host cells with:

- (i) DNA encoding a modified ecdysone receptor, wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and
- (ii) a DNA construct encoding said recombinant product under the control of a response element, wherein said response element (a) has about 12-20 base pairs, (b) binds to said modified ecdysone receptor, and (c) does not bind to farnesoid X receptor (FXR);

growing said host cells in suitable media; and

inducing expression of said recombinant product by introducing into said host cells one or more ligands for said modified ecdysone receptor, and optionally a silent partner for said modified ecdysone receptor.

47. (Amended) A method according to claim 1, wherein said silent partner is present.

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48. (Amended) A method according to claim 47 wherein said silent partner is ultraspiracle.

49. (Amended) A method according to claim 1 wherein said modified ecdysone receptor does not bind to endogenous response elements.

50. (Amended) A method for modulating the expression of an exogenous gene in an isolated cell containing:

- (i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and
- (ii) a modified receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;

said method comprising providing to the cell an effective amount of one or more ligands for said modified receptor; wherein said one or more ligands are not normally present in the cell; and wherein said one or more ligands are not toxic to said cell.

51. (Amended) A method according to claim 50, wherein said silent partner is present.

52. (Amended) A method according to claim 51, wherein said silent partner is ultraspiracle.

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54. (Amended) A method according to claim 51, wherein said silent partner is RXR.

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58. (Amended) A method according to claim 50 wherein the DNA-binding domain of said modified receptor is derived from a nuclear receptor.

59. (Amended) A method according to claim 50 wherein said activation domain is derived from a nuclear receptor.

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61. (Amended) A method according to claim 50, wherein said ecdysone response element does not bind to farnesoid X receptor (FXR).

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67. (Amended) A method of inducing the expression of an exogenous gene in an isolated cell containing:

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- (i) a DNA construct comprising an exogenous gene under the control of an ecdysone response element,
 - (ii) DNA encoding a modified receptor under the control of an inducible promoter, wherein said modified receptor, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;
 - (iii) one or more ligands for said modified receptor;

said method comprising subjecting said cell to conditions suitable to induce expression of said modified receptor.

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68. (Amended) A method of inducing expression of an exogenous gene in an isolated cell containing a DNA construct containing said exogenous gene under the control of an ecdysone response element, said method comprising introducing into said cell:

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a modified receptor, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and

one or more ligands for said modified receptor,

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wherein said modified receptor, in combination with a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, activating transcription therefrom.

69. (Amended) A method for the expression of a recombinant product detrimental to isolated host cells, said method comprising:

transforming suitable isolated host cells with:

- (i) a DNA construct encoding said recombinant product under the control of an ecdysone response element, and
- (ii) DNA encoding a modified receptor, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and

growing said host cells in suitable media; and

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inducing expression of said recombinant product by introducing into said host cells one or more ligands for said modified receptor, and optionally a silent partner for said modified receptor.

70. (Amended) A method for modulating the expression of an exogenous gene in an isolated cell containing:

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- (i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and
 - (ii) a modified receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element wherein said modified receptor has substantially no constitutive activity in mammalian cells, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;

said method comprising providing to the cell an effective amount of one or more ligands for said modified receptor; wherein said one or more ligands are not normally present in the cell; and wherein said one or more ligands are not toxic to said cell.

71. (Amended) A method for modulating the expression of an exogenous gene in an isolated cell containing:

- (i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and
- (ii) a modified ecdysone receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, wherein said modified receptor has an altered DNA

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binding specificity relative to the wildtype receptor from which it is derived, and wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said ecdysone response element; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;

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said method comprising providing to the cell an effective amount of one or more ligands for said modified ecdysone receptor; wherein said one or more ligands are not normally present in the cell; and wherein said one or more ligands are not toxic to said cell.

72. (Amended) A method for modulating the expression of an exogenous gene in a mammalian subject containing:

- (i) a modified ecdysone receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to a response element, and wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said response element; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and
- (ii) a DNA construct comprising said exogenous gene under the control of said response element, wherein said response element (a) has about 12-20 base pairs, (b) binds to said modified ecdysone receptor, and (c) does not bind to farnesoid X receptor (FXR);

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said method comprising providing to said subject an effective amount of one or more ligands for said modified ecdysone receptor; wherein said one or more ligands are not normally present in said subject; and wherein said one or more ligands are not toxic to said subject.

73. (Amended) A method of inducing the expression of an exogenous gene in a mammalian subject containing:

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- (i) DNA encoding a modified ecdysone receptor under the control of an inducible promoter, wherein said modified ecdysone receptor, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to a response element, and wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said response element; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;
 - (ii) a DNA construct comprising said exogenous gene under the control of said response element, wherein said response element (a) has about 12-20 base pairs, (b) binds to said modified ecdysone receptor, and (c) does not bind to farnesoid X receptor (FXR); and
 - (iii) one or more ligands for said modified ecdysone receptor;

said method comprising subjecting said subject to conditions suitable to induce expression of said modified ecdysone receptor.

74. (Amended) A method of inducing expression of an exogenous gene in a mammalian subject containing a DNA construct containing said exogenous gene under the control of a response element, wherein said response element (a) has about 12-20 base pairs, (b) binds to a modified ecdysone receptor, and (c) does not bind to farnesoid X receptor (FXR), said method comprising introducing into said subject:

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a modified ecdysone receptor, wherein said modified ecdysone receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid, (b) a DNA-binding domain obtained from a DNA-binding protein, which binds to said response element; and (c) an activation domain of a transcription factor, wherein at least one of said DNA-binding domain or said activation domain is not obtained from a native ecdysone receptor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and

one or more ligands for said modified ecdysone receptor,

wherein said modified ecdysone receptor, in combination with a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said response element, activating transcription therefrom.

75. (Amended) A method for modulating the expression of an exogenous gene in a mammalian subject containing:

- (i) a DNA construct comprising said exogenous gene under the control of an ecdysone response element; and
- (ii) a modified receptor which, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;

said method comprising providing to said subject an effective amount of one or more ligands for said modified receptor; wherein said one or more ligands are not normally present in said subject; and wherein said one or more ligands are not toxic to said subject.

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76. (Amended) A method of inducing the expression of an exogenous gene in a mammalian subject containing:

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- (i) a DNA construct comprising an exogenous gene under the control of an ecdysone response element,
- (ii) DNA encoding a modified receptor under the control of an inducible promoter, wherein said modified receptor, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein;
- (iii) one or more ligands for said modified receptor;

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said method comprising subjecting said subject to conditions suitable to induce expression of said modified receptor.

77. (Amended) A method of inducing expression of an exogenous gene in a mammalian subject containing a DNA construct containing said exogenous gene under the control of an ecdysone response element, said method comprising introducing into said subject:

a modified receptor, wherein said modified receptor does not bind to endogenous response elements, and wherein said modified receptor comprises: (a) a ligand binding domain that binds to an ecdysteroid; (b) a DNA-binding domain derived from a DNA-binding protein, wherein said DNA-binding domain binds to said ecdysone response element but not to endogenous response elements; and (c) an activation domain of a transcription factor, with the proviso that when said activation domain is derived from a glucocorticoid receptor, said DNA-binding domain is not derived from a glucocorticoid receptor or an E. coli LexA protein; and one or more ligands for said modified receptor, .

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wherein said modified receptor, in the presence of a ligand therefor, and optionally in the further presence of a silent partner therefor, binds to said ecdysone response element, activating transcription therefrom.
